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Spinaxinus (Bivalvia: Thyasiroidea) from sulfide biogenerators in the Gulf of Mexico and hydrothermal vents in the Fiji Back Arc: chemosymbiosis and taxonomy

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SUMMARY: Two new species of the thyasirid genus *Spinaxinus* (*S. emicatus* Oliver n. sp. and *S. phrixicus* Oliver n. sp.) are described from the Gulf of Mexico and the southwest Pacific, respectively. Both are compared with the type species of the genus, the eastern Atlantic *S. sentosus* Oliver and Holmes, 2006. Living specimens from the Gulf of Mexico were retrieved from artificial sulfide bio-generators on the upper Louisiana Slope. Gill morphology and molecular markers from the symbiotic bacteria confirm that *Spinaxinus* is chemosynthetic and that the chemoautotrophic bacteria are related to sulfide oxidizing Gammaproteobacteria. Living specimens from the southwest Pacific were retrieved from hydrothermal vent sites in the Fiji and Lau Back Arc Basins. In the Atlantic *Spinaxinus* is now recorded from two anthropogenic situations and appears to be generally absent from natural cold seep sites and not yet recorded at any hydrothermal sites. The primarily anthropogenic distribution of *Spinaxinus* in the Atlantic is discussed with reference to the natural hydrothermal vent habitat of the Pacific *S. phrixicus*.

Keywords: *Thyasiroidea,* taxonomy, chemosymbiosis, anthropogenic settings, hydrothermal vents.

RESUMEN: *Spinaxinus* (Bivalvia: Thyasiroidea) de bio-generadores artificiales de sulfuro situados en el Golfo de Méjico y en fuentes hidrotermales de las Islas Fiji: quimiosimbiosis y taxonomía. – En este trabajo se describen dos especies nuevas de tisárido del género *Spinaxinus* (*S. emcatus* Oliver n. sp. y *S. phrixicus* Oliver n. sp.) encontradas respectivamente en el Golfo de Méjico y en el sureste del Pacífico. Se comparan estas dos especies nuevas con la especie tipo del género, *S. sentous* Oliver y Holmes, 2006 descrita en el Este del Atlántico. Para describir estas dos especies, se observaron ejemplares vivos recolectados sobre bio-generadores artificiales de sulfuro situados en la parte alta de la plataforma continental de Louisiana, en el Golfo de Méjico. Las observaciones realizadas de las branquias de *Spinaxinus* y la caracterización genética de las bacterias simbiontes en estos ejemplares confirmaron que *Spinaxinus* es un género quimiosintético que contiene bacterias quimioautótrofas cercanas a las Gammaproteobacterias responsables de la oxidación del sulfuro. También se recolectaron ejemplares vivos de fuentes hidrotermales situadas en las Islas Fiji y en 'Lau Back Arc Basins' ambas localizadas en el Pacífico suroccidental. La especie atlántica de *Spinaxinus* se encontró en dos tipos de sustratos artificiales mientras que parece que esta especie no se encuentra en ambientes naturales equivalentes como serían las surgencias frías y las fuentes hidrotermales. La distribución aparentemente limitada de la especie atlántica se discute en relación con la distribución de *S. phrixicus* en las fuentes hidrotermales del Pacífico.

Palabras clave: *Thyasiroidea,* taxonomía, quimiosimbiosis, sustratos artificiales, fuentes hidrotermales.

INTRODUCTION

The genus *Spinaxinus* was erected from specimens collected from the wreck of a cargo vessel that sunk off the Atlantic coast of Spain in 1979 and lay at 1160 m. During salvaging from 1991-1992 a community of organisms, including vestimentiferan worms and bivalves, was found living on the cargo of sisal bales, sunflower seeds and beans (Dando *et al.* 1992). The thyasirids collected were described as *Spinaxinus sentosus* Oliver and Holmes, 2006 and were inferred to be chemosymbiotic from the substrate and associated fauna (Oliver and Holmes 2006). The associated fauna included the vestimentiferan, *Lamellibrachia barhami* Webb, 1969 and the mussel *Idasola* (Dando *et al.* 1992). The state of preservation was not sufficient to carry out an electron microscopy study or for molecular analysis. To date this genus has only been found in this artificial environment except for a single juvenile shell taken from a mud volcano in the Gulf of Cadiz (Oliver *et al.* 2011).

This paper primarily reports upon a newly discovered population of a *Spinaxinus* retrieved from artificial sulfide bio-generators in the Gulf of Mexico (Carney *et al.* 2010). The Gulf of Mexico specimens are well preserved and have allowed both morphological and molecular studies. Comparisons with the eastern Atlantic *S. sentosus* are restricted to shell morphology due to poor preservation. During these investigations a third species of *Spinaxinus* has been recognized from the Fiji Back Arc and Lau Basins. This is the species referred to by Taylor *et al.* (2007) as "*Thyasira* sp. (vent species)" and although represented by a single complete adult specimen it is described here.

MATERIALS AND METHODS

Gulf of Mexico sulfide bio-generators

Rationale

The program of device development and deployment was inspired by the report of lamellibrachid tubeworms in the decomposing agricultural cargo of the shipwreck *Francois Vieljeux* (Dando *et al.* 1992). If organic decomposition in smaller devices could similarly produce sufficient sulfide to attract and support sulfide-based chemosynthesis, then there would be an experimental tool for the study of seep community colonization and dispersal. In 1996 deployment of prototype devices in the Gulf of Mexico began, resulting in their colonization by 555 lamellibrachid tubeworms after five years (Carney unpublished data). At that time molluscs were not encountered in those prototypes. More extensive deployment has taken place since that time.

Sulfide biogenerator construction

The primary components of the devices (Fig. 1) consist of a bed of pressed alfalfa pellets sold as rabbit food (Purina Rabbit Chow Complete). This chow was comprised of compressed 4×10 mm pellets of dehydrated alfalfa with lesser amounts of other grain and soybean products. The pellets are an inexpensive source of labile plant carbon which are safe, easy to handle and unlikely to attract scavengers as a meat product might. To promote rapid initial development of a sulfide producing microbiota, sodium sulfate (Na_2SO_4) was added to delay the onset of sulfate limitation. The bed of pellets was topped by a 12-cm layer of carbonate shells intended to simulate the hard substrate favoured for settlement by tubeworms in natural seep systems. The shells used, of the marsh clam *Rangia cuneata*, are available in fairly large quantities, having once been used for road paving in some coastal areas of the United States.

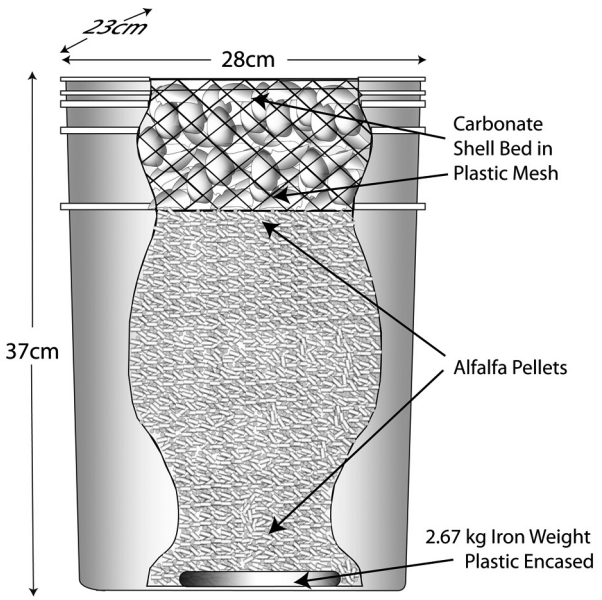

FIG. 1. – Diagram of the sulfide bio-generator deployed in the Gulf of Mexico.

Two different plastic pails were used. Deployments made in August 2002 used 15-L opaque pails with a square 23×23 cm mouth and a height of 33 cm. Deployments made in August 2003 used 20-L translucent pails with a rectangular mouth measuring 23×28 cm and a height of 37 cm. A plastic encased 2.67-kg iron weight was placed at the bottom of the pail to overcome initial positive buoyancy. The pail was then filled to within 12 cm of the top with rabbit chow. Five hundred grams of dry sodium sulfate was then added. The plastic mesh container of shell material was then placed on top of the rabbit chow layer and secured by drilling four holes through the edge of the mouth and placing an electrical tie through the pail and the mesh.

Deployment and recovery

The discovery of *Spinaxinus* was fortuitous in these tubeworm colonization experiments. The

specimens were found in the shell layer, which had become filled with detritus of decomposed alfalfa pellets. The devices containing *Spinaxinus* were recovered in August 2006 by the submersible Johnson Sealink II operated by the Harbor Branch Oceanography Institution. The holotype was found in a device deployed in August 2002 on mud bottom in the vicinity of a chemosynthetic community and a brine pool. The paratypes were found in deployments made in August 2003 on a mud bottom with no known seep communities in the immediate vicinity. Sulfide and pH microprobe profiles from the biogenerators confirmed that the intended hydrogen sulfide generation was active and that they were functioning as artificial sulfidic systems (Bertics and Ziebis unpublished data).

Molecular characterization of host and associated bacteria

DNA was extracted from gill tissue of three specimens using DNA Blood and Tissue Kit (QIAGEM, CA). Host gene encoding 28S rRNA was amplified using the primer combination LSU-900f/LSU-1600r (Williams *et al.* 2004). Bacterial genes encoding 16S rRNA and adenosine 5'-phosphosulfate (APS) reductase were amplified using the primer combinations 27F/1492R (Lane 1991) and APRA_1FW/APRA_5RV (Meyer and Kuever 2007), respectively. PCR products were sequenced directly at GATC Biotech (Constanz, Germany); as sequence chromatograms displayed no ambiguity such as double peaks, no additional cloning step was added for 16S rRNA and APS

Sequences were compared with sequences available in Genbank using BLAST (http://www.ncbi.nlm.nih.gov) (Altschul *et al*. 1997). The 16S rRNA phylogenetic relationships were reconstructed using maximum likelihood (ML) with the PHYLIP package (Felsenstein 1995).

Southwest Pacific material

Material of *Spinaxinus* from the SW Pacific was that used by Taylor *et al*. (2007) and housed in the Swedish Museum of Natural History. It originates from the TUI MALILA Expedition (TUIM) during 2005 of the Fiji Back Arc and Lau Basins aboard the RV Melville and utilizing the ROV Jason. The specimens came from two sites, the White Lady Vent and Hine Hina Vent; background data on these sites can be viewed at http://www.interridge.org/irvents/content/white-lady and http://www.interridge.org/irvents/content/hine-hina.

Shell morphology

Shell images were made using a computer-aided digital photographic system powered by AutoMontage™ and by scanning electron microscopy with a JEOL Neoscope.

Gross anatomy

The specimens had previously been fixed in and stored in 100% ethanol, subsequently stained with rose bengal or methylene blue. The shell was dissected away from the soft tissues and the anatomy was viewed and dissected from the left side. Images were made using a computer-aided digital photographic system powered by AutoMontage™.

Critical Point Drying and SEM examination

For SEM examination the tissues were dissected from the shell and the ctenidia were excised and sliced transversely with a razor blade into several pieces. The tissue pieces were dehydrated in 100% ethanol and critical point dried. The specimens were then mounted on stubs, sputter coated with gold and examined using a Jeol Neoscope scanning electron microscope.

SYSTEMATICS

Class BIVALVIA Linnaeus, 1758
Subclass HETERODONTA Neumayr, 1884
Order VENEROIDA H. and A. Adams, 1856
Superfamily THYASIROIDEA Dall, 1900
Family THYASIRIDAE Dall, 1900
Genus *Spinaxinus* Oliver and Holmes, 2006

Type species: *Spinaxinus sentosus* Oliver and Holmes, 2006 OD

Spinaxinus emicatus Oliver, n. sp.

Type material. Holotype 1 sp., Louisiana slope, Gulf of Mexico, 27°44.1292´N 91°15.2858´W, 643 m, NMW.Z.2013.1.1; Paratypes 4 sp., as holotype; 4 sp., Louisiana slope, Gulf of Mexico, 27° 45.9164´N 91° 06.7308´W, 584 m, NMW.Z.2013.1.2 and USNM All leg. R. Carney.

Comparative material. Spinaxinus sentosus, Holotype and paratypes, hold of the cargo ship *Francois Vieljeux* approx. 30 miles west of Vigo, Spain, 42°7.95´N 9°26.95´W, 1160 m. 1992. NMW.Z.2002.108.1/2. *Thyasira oleophila*, Louisiana slope, Bush Hill GC-185, 27°46.941´N 91°30.479´W, 1738 feet (529 m), FMNH307755. *Thyasira conia*, Syntypes, San Juan Harbour, Porto Rico, 310 fathoms (567 m). USNM 108949.

Diagnosis. A moderately large thyasirid with a spicate periostracum, uncalcified periostracal spines becoming black in larger individuals. Compressed, subcircular in outline, beaks close to the midline, posterior sulcus weak, posterior area relatively wide. Residual anterior protractor muscle scars absent. Ctenidia with laminar filaments, abfrontal region expanded, lined with bacteriocytes with a glycocalyx.

Description (Figs 2-5). Shell to 21.7 mm in length, holotype 19.4 mm (Fig. 2, Table 1). Thin, fragile. Equivalve. Not inflated. Beaks not elevated, prosogyrate. Outline variable (Fig. 3), height and length approximately equal but some longer than high and some higher than long; approximately subcircular, most expanded poste-

Table 1. – Shell measurements.

	Length	Height	Tumidity
Holotype	19.4	19.6	4.8
Paratype	21.7	20.3	6.4
Paratype	16.1	16.2	4.0
Paratype	14.0	14.8	3.7
Paratype	13.7	13.6	3.5
Paratype	15.8	13.4	4.4
Paratype	18.5	17.6	5.7
Paratype	11.7	10.7	2.8
Paratype	9.5	8.8	2.1

riorly and narrower anteriorly; posterior dorsal margin long, curved, marking the very narrow and shallow escutcheon; posterior margin subtruncate weakly sinuate; ventral and anterior forming a continuous curve to meet a long lunule (Fig. 3C). Escutcheon very narrow, auricle absent (Fig. 3B, C). Posterior sinus shallow but distinct, setting off a relatively wide posterior area. Ligament opisthodetic on a deeply sunken resilifer just over half the length of the escutcheon. Hinge teeth absent. Sculpture of irregular commarginal lines and growth stops, microsculpture weakly rippled (Fig. 4D). Periostracum persistent, greyish to olive-buff in colour, minutely spicate (Fig. 5A); in larger shells on the central areas (Fig. 4B) the uncalcified spines are short dark, almost black, in colour, on the margins (Fig. 4C, 5B) the spines are dense, long with longitudinal strengthening ridges (Fig. 5C). Internal muscle scars apparent (Fig. 2D), anterior adductor scar long and almost twice the area of the posterior scar, separation from the pallial line slight; posterior adductor scar narrow, partly on the posterior area; pallial line thick, entire.

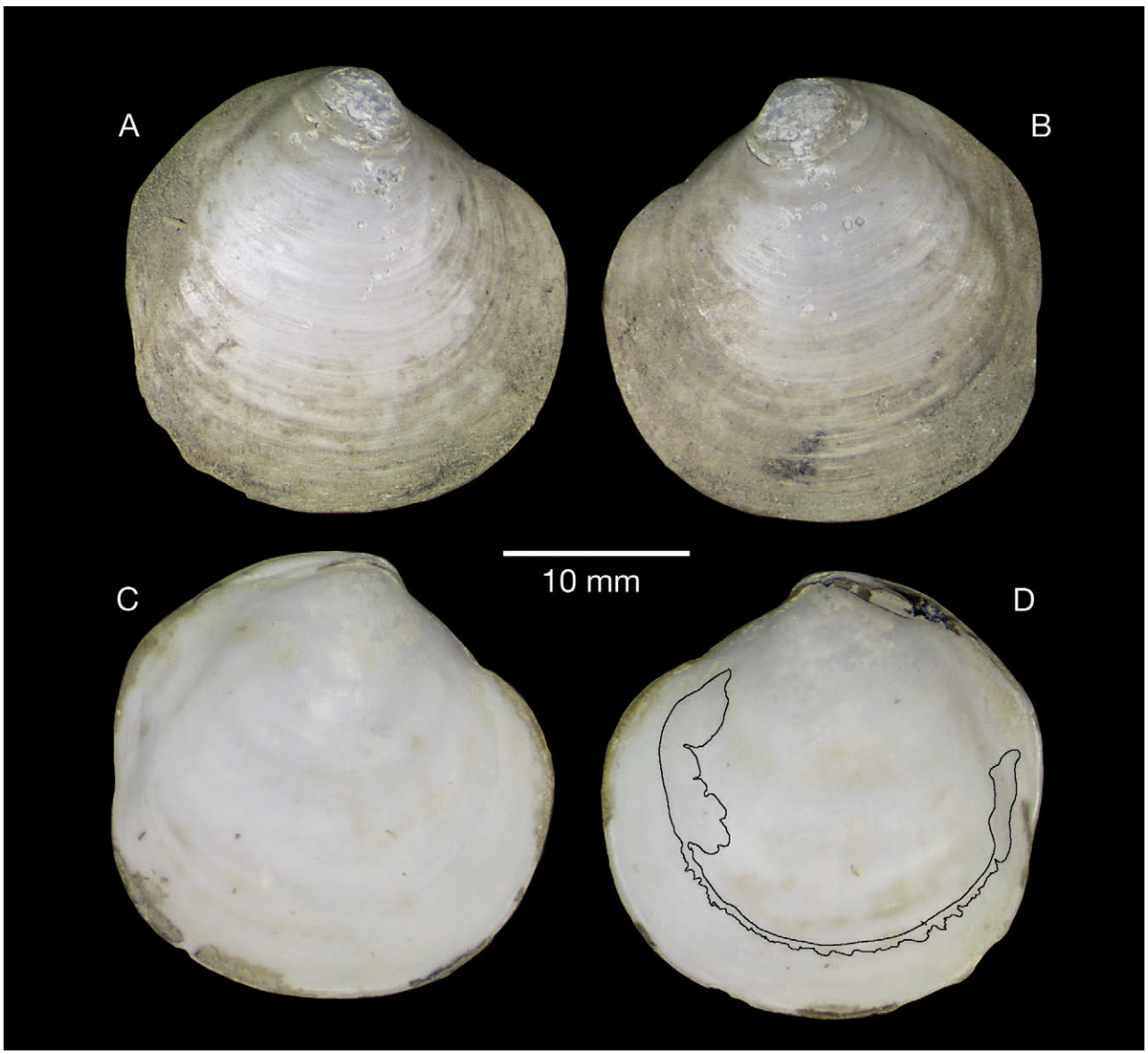

Fig. 2. – *Spinaxinus emicatus n. sp.*, Holotype, NMW.Z.2013.1.1. A and D, right valve, internal view with outline of muscle scars superimposed; B and C, left valve.

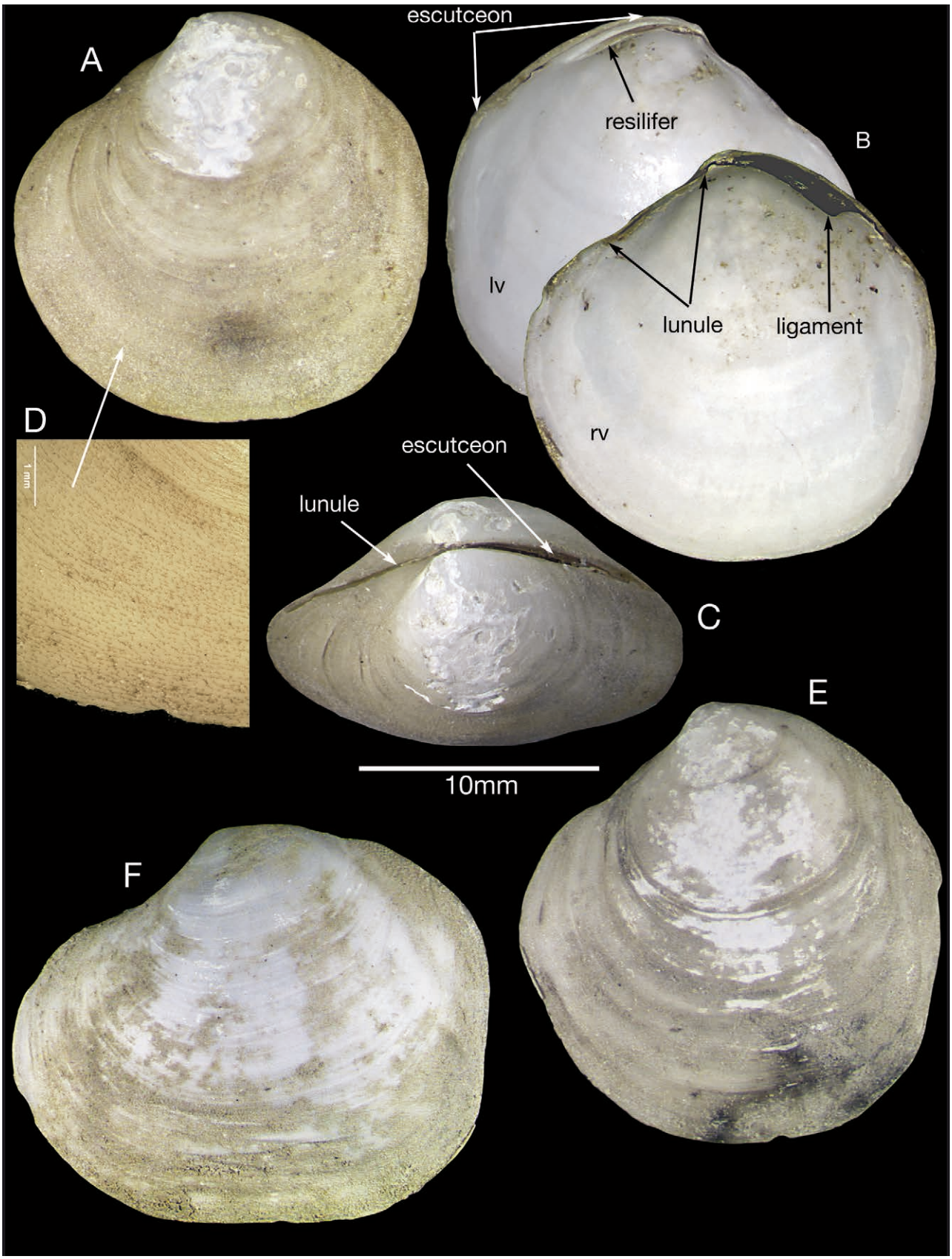

FIG. 3. – *Spinaxinus emicatus*, Paratypes to show variation in outline.

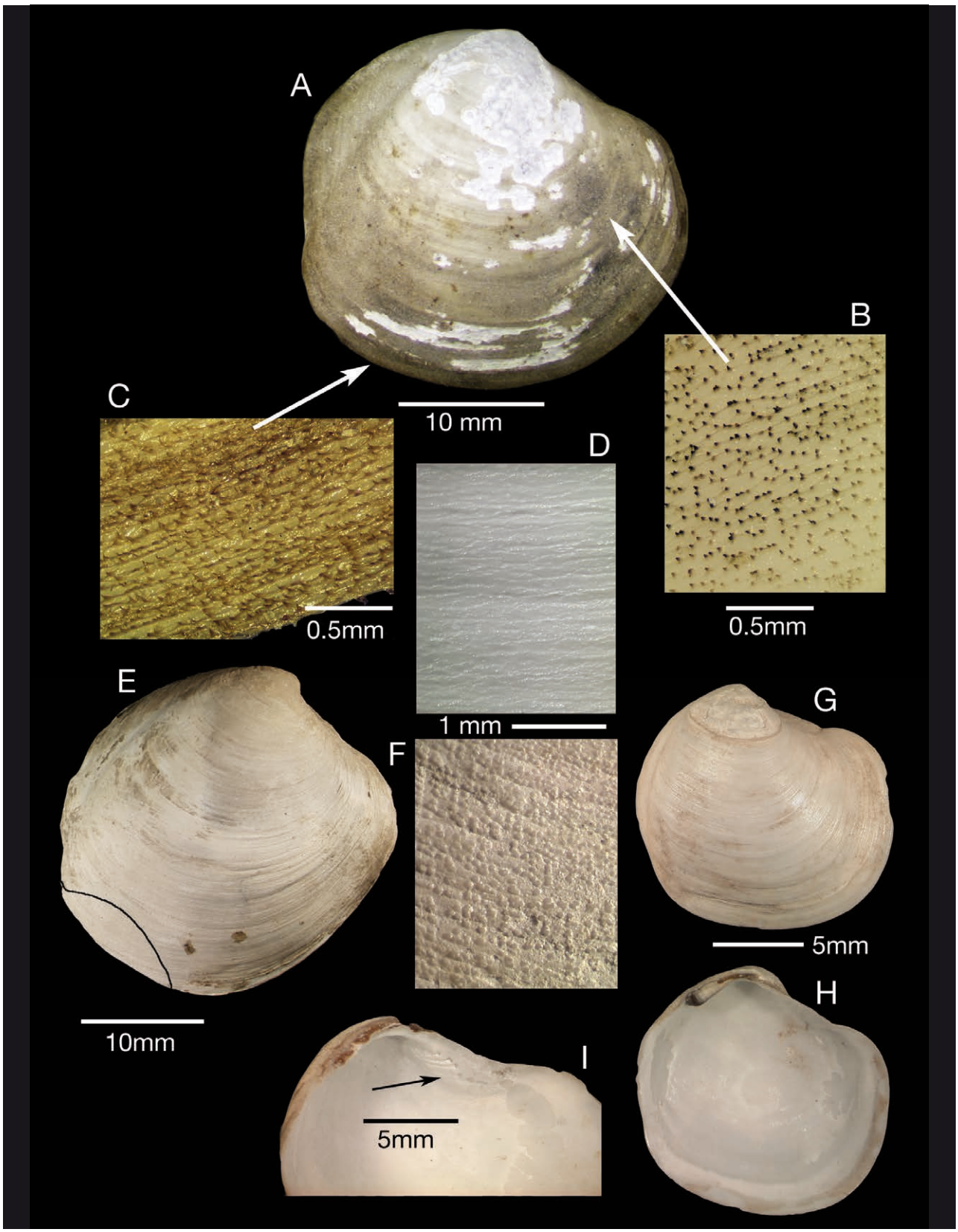

FIG. 4. – A-D *Spinaxinus emicatus*: A, right valve of largest specimen; B, periostracal spines on middle area; C, periostracum on margins; D, shell surface with periostracum removed. E-F, *Thyasira oleophila*, Louisiana slope: E, a right valve; F, surface detail. G-I, *Spinaxinus sentosus*, off Vigo, Spain: G-H, holotype; I, detail showing residual pedal retractor scars.

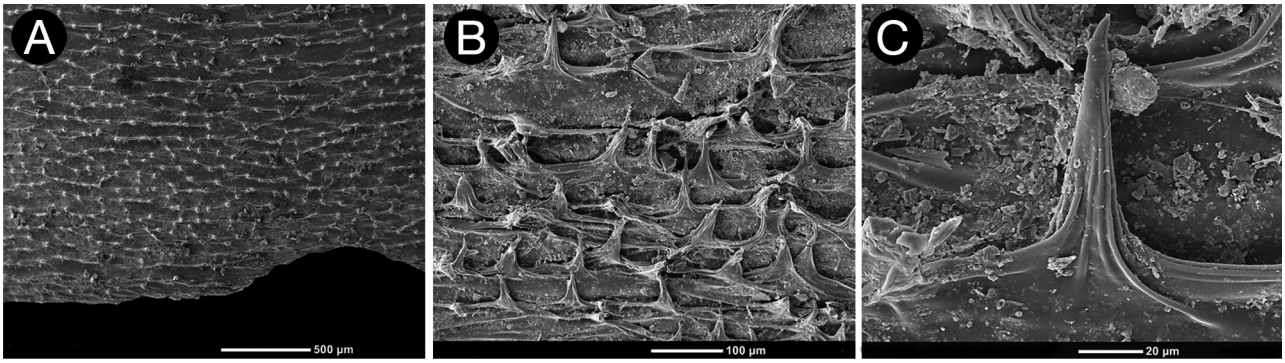

FIG. 5. – Scanning electron micrographs at progressive magnifications of the periostracum of *Spinaxinus emicatus*.

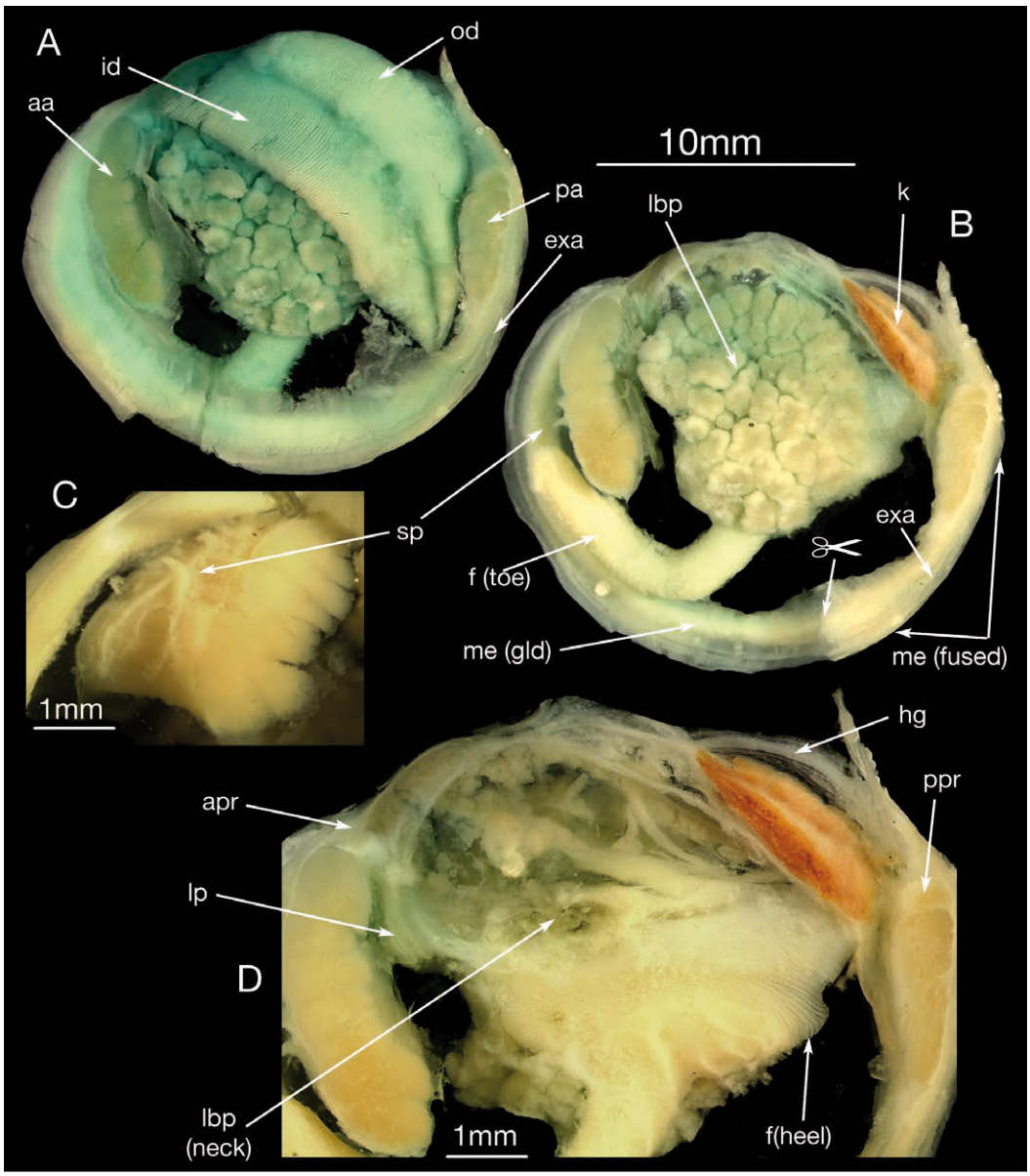

FIG. 6. – Gross anatomy of *Spinaxinus emicatus*. A, with mantle removed; B, with mantle edge and ctenidium removed; C, sensory papilla on the outer face of the anterior adductor muscle; D, with lateral pouch removed and partial dissection of the visceral mass.aa, anterior adductor muscle; apr, anterior pedal retractor muscle; exa, exhalant aperture; f, foot; hg, hind gut; id, inner demibranch; k, kidney; lbp, lateral body pouch; lp, labial palp; me (gld), glandular area of mantle edge; me, mantle edge; od, outer demibranch; pa, posterior adductor muscle; ppr, posterior pedal retractor muscle; sp, sensory papilla.

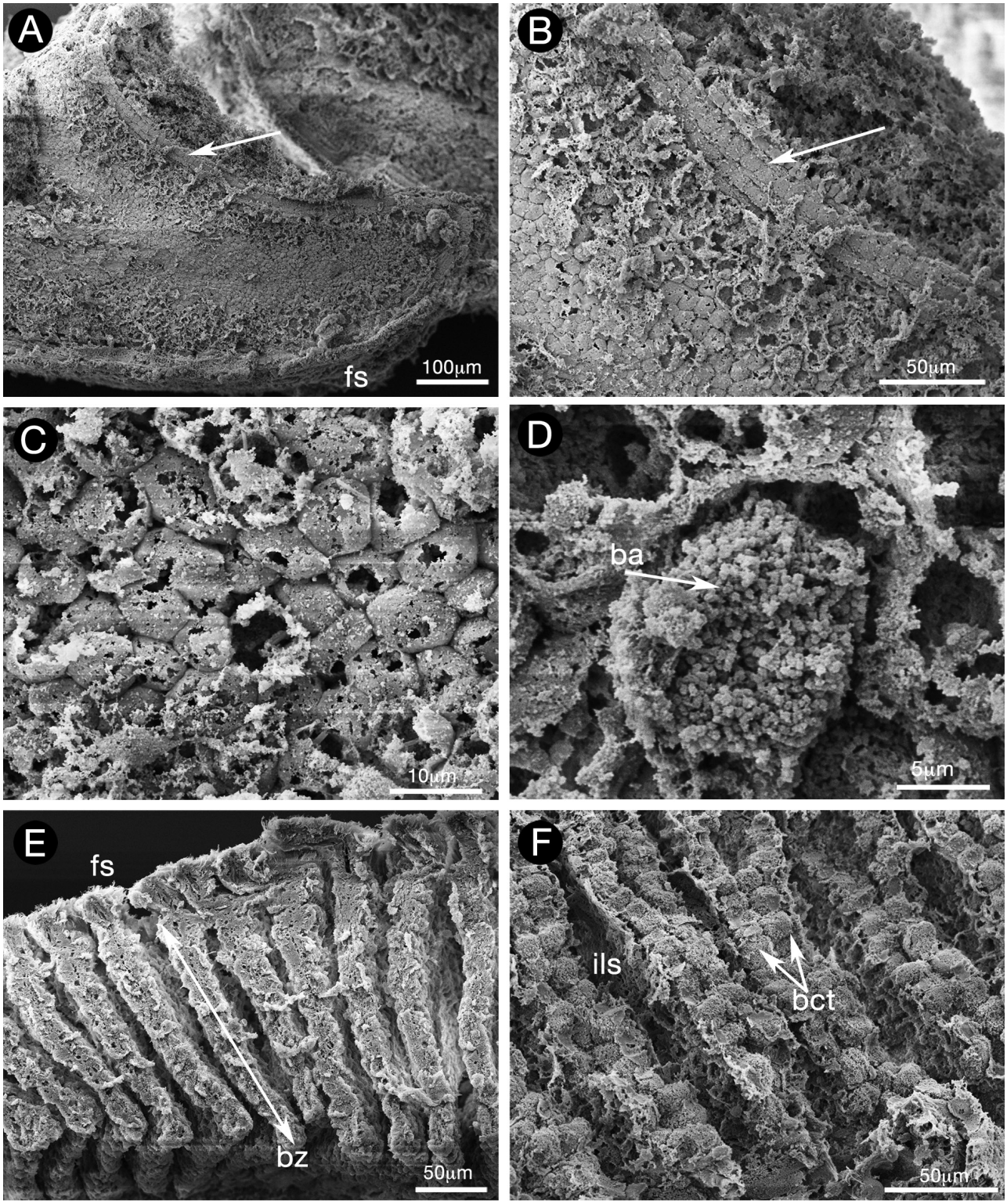

FIG. 7. – Scanning electron micrographs of the ctenidium of *Spinaxinus emicatus*. A, lateral face of a lamellar filament, (fs) frontal surface, (arrowed) inner surface with double layer of cuboidal cells arrowed. B, detail of A showing polygonal pattern of bacteriocytes. C, surface of bacteriocytes with glycocalyx coating. D, single bacteriocyte with glycocalyx eroded and revealing bundle of bacteria (ba). E, transverse slice of a series of filaments showing greatly elongated bacteriocyte zone (bz). F, broken abfrontal edges showing large bacteriocytes (bct), interlamellar spaces (ils).

Gross anatomy (Fig. 6). Mantle edge free except for a short siphonal opening posteriorly, inner fold thick and glandular (Fig. 6B). Anterior adductor muscle large, elongate with a sensory papilla at one third its

length on the outer face (Fig. 6C); posterior adductor muscle narrow; anterior and posterior pedal retractor muscles attached above and next to their respective adductor muscles (Fig. 6A). Foot vermiform (Fig. 6B), long; heel indistinct (Fig. 6D). Ctenidia with outer and inner demibranchs (Fig. 6A). Labial palps narrow lacking sorting ridges (Fig. 6D). Lateral body pouches large, tubules irregularly cuboidal (Fig. 6B), joined to stomach ventrally (Fig. 6D). Stomach small, details destroyed by removal of lateral pouch, mid-gut and hind gut present. Kidneys large, bright orange in colour due to dense granular contents (Fig. 6B, D).

Gill morphology (Fig. 7). The ctenidia are composed of both outer and inner demibranchs, the outer slightly shorter but extending more dorsally to expose the majority of the inner demibranch. The filaments are reflected with their abfrontal regions greatly extended to form a lamella Fig. 7A). The outer surface is ciliated (Fig 7A, E, fs) and there is a narrow intermediary zone in front of a greatly extended abfrontal zone (Fig 7E, bz arrowed). The inner edge of each lamella is lined by a double row of large cuboidal bacteriocytes (Fig. 7A,B, arrowed). The abfrontal zone of each lamella is lined on both sides by a layer of polygonal bacteriocytes (Fig. 7F, bct), each covered by a glycocalyx (Fig. 7C). Where the glycocalyx is damaged or broken away a dense bundle of bacteria is seen filling each cell (Fig. 7D, ba). The bacteria are small, and although preservation is not good, they appear to be ovoid and less than 1 μm in diameter.

Molecular characterization. All three specimens examined shared the same partial 28S rRNA-encoding sequence that showed 99% similarity (704/707 bp) with *Thyasira* sp. STW-2006 (AM392436), a vent species from Fiji Back Arc Basin. Unfortunately, no molecular data are available for *Spinaxinus sentosus* and its relationship with this new species cannot be addressed.

Etymology. From the Latin *emico*, "to appear suddenly" (from Brown 1956); referring to the unexpected appearance in the colonization experiments.

Differential diagnosis. Species discrimination in thyasirids has been based primarily on shell characters but can be difficult due to the relatively few shell characters available, as a consequence of the lack of a complex hinge dentition and shell sculpture. However, shell morphology is the basis of most species descriptions and remains widely used (Oliver and Killeen 2002, Zelaya 2010, Keunig 2010, Coan and Valentich Scott 2012, Oliver and Drewery 2013). Only combined morphology and molecular data using proper markers on large numbers of specimens from both sides of the Atlantic might allow unambiguous delimitation of species, although one must also keep in mind the pitfalls of molecular species delimitation (sampling bias and inter-specific divergence threshold). Future modifications of our conclusions may come with additional data from larger samples and from molecular data such as those demonstrated by Lorien *et al.* (2010) for bathymodiolines. However, we do not have material of the type species *S. sentosus* fixed appropriately for molecular study, and given the transient nature of the type locality it is unlikely that such data will ever be forthcoming; therefore, we must resort to comparative shell morphology. Variation in bivalve shell morphology has often been regarded as allometric or ecophenotypic. In thyasirids allometric changes occur early in the growth stages over the first 1-3 mm (Oliver and Killeen 2002) and are therefore unlikely to account for the differences seen here between *S. sentosus* and *S. emicatus*. Small differences in shell form have often been dismissed as ecophenotypic and this is certainly true for many epifaunal bivalves (Oliver and Holmes 2006) but less so for infaunal taxa, in which small differences are now being found to be significant as species characters (Taylor and Glover 2005).

The spicate periostracum is characteristic of the genus *Spinaxinus* and the only other known Atlantic species in the genus is *S. sentosus* (Fig. 4G-I) from off Spain. A third species from the Southwest Pacific is described and differentiated below.

In both Atlantic species the marginal spines are long, with longitudinal reinforcing ridges, but in *S. emicatus* the spines over the median area are persistent and prominent, becoming almost black in colour (Fig. 4B). The two species differ in shell shape, with *S. sentosus* being more oblong, so the length to height ratio is greater, and more oblique because the length to anterior length ratio is lower (Fig. 8A); the escutcheon is proportionately shorter and the posterior area is narrower (Fig. 8C). In *S. sentosus* accessory anterior pedal retractor scars are present (Fig. 4I) but they are absent in *S. emicatus.* In shell morphology alone the Gulf of Mexico and eastern Atlantic samples can be regarded as distinct species.

The conclusion that the Gulf of Mexico and eastern Atlantic samples represent different species is further supported by the biogeography of thyasirids, which have no species in common between the two regions. The Gulf of Mexico is relatively poor in thyasirids, with the deep-water taxa *T. oleophila* Clarke, 1989, *T. conia* Dall and Simpson, 1901, *Conchocele disjuncta* Gabb, 1866 (Boss 1967) and an undescribed species of *Axinulus* (Oliver unpublished), all restricted to the Caribbean. *Thyasira oleophila* has been collected from nearby oil seeps on the Louisiana slope but does not have a spicate periostracum and its shell surface is distinctly pustulose (Figs 4E, F).

Thyasira conia Dall and Simpson, 1901 is a small species (to 6 mm) and is described as having a dust-like granular surface. This species is only known from the three syntypes and on examination the surface is granular but not with a spicate periostracum.

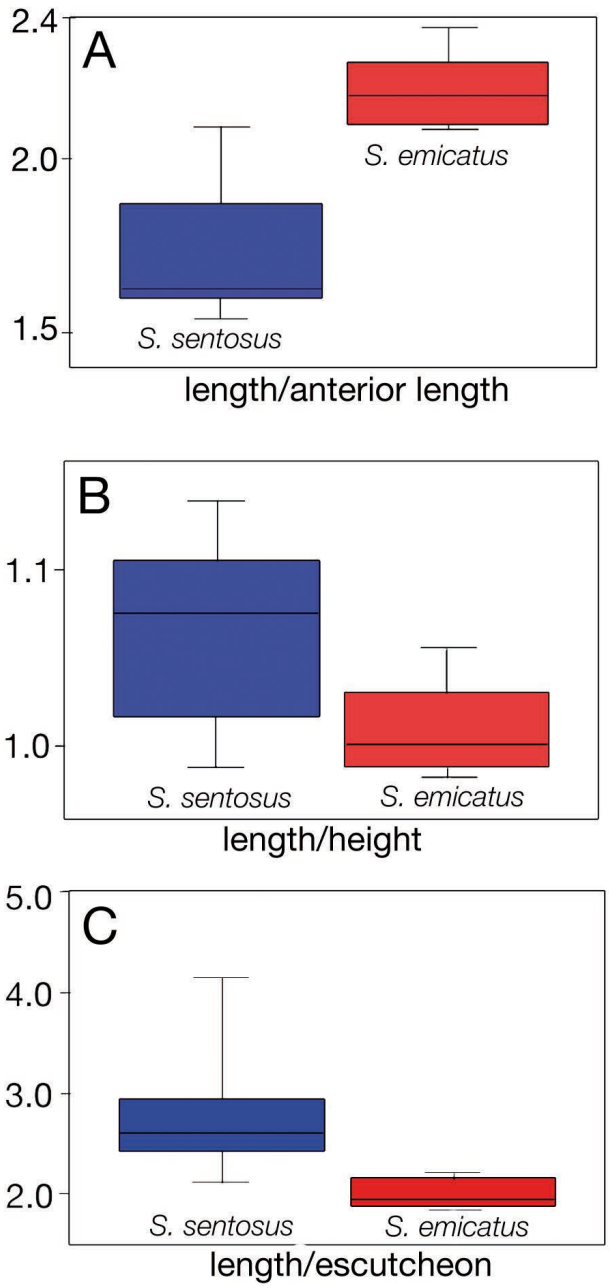

FIG. 8. – Box plots showing relative ratios of three shell dimensions for *Spinaxinus emicatus* and *S. sentosus*. A, length to anterior length; B, length to height; C, length to escutcheon length.

There is debate about the systematics of the shallow water *T. trisinuata* (d'Orbigny, 1853), which is believed by some to extend its range along the whole of the Atlantic coast of North America (Payne and Allen 1991).

Co-occurring with *Spinaxinus* in the Gulf of Mexico is the vestimentiferan *Lamellibrachia luymesi* van der Land and Nørrevang, 1975, a species not yet recorded from the eastern Atlantic and not that recently discovered in the Mediterranean and described as *L. anaximandri* Southward *et al.* 2011. The initial identification of the *Lamellibrachia* from the *Francois Vieljeux* as the Pacific species *L. barhami* could not be confirmed from molecular data and it is more likely that it was the same as the Mediterranean species (Eve Southward pers. comm.). The issue of amphi-Atlantic distributions of seep and vent species therefore remains unresolved, and because of the morphological difference we regard the Gulf of Mexico *Spinaxinus* as separate from *S. sentosus* in the NE Atlantic.

Spinaxinus phrixicus Oliver n. sp.

Type material. Holotype, 1 sp., RV Melville/ROV Jason cruise TUIM06MV, White Lady Vent Field, Fiji Back Arc Basin, 16.99°S 173.914°E, 1977 m, 31 May 2005. Hydrothermal seep with patches of Alviniconcha, SMNH – 78311.

Other material. 1 fragment with tissue as holotype, cross reference to Genbank AM392452.1 and AM392436.1, SMNH – 78311. 1 minute sp,1 mm, as holotype, SMNH – 78558. 2 sh., RV Melville/ROV Jason cruise TUIM06MV, Hine Hina, Lau Back Arc Basin, 22.534°S 176.71001°E, 1847 m, Hydrothermal seep, in gravel with bacterial mat, SMNH – 78277.

Comparative material examined. As under *S. emicatus* above.

Diagnosis. A medium-sized thyasirid with a fine spicate periostracum. Compressed, subcircular in outline with a weakly defined posterior area. Shell sculpture of few increasingly widely spaced commarginal low ridges.

Description. Shell (Figs 9A-B) length, 8.7 mm; height, 8.2 mm; tumidity, 4.1 mm. Thin, fragile. Equivalve. Not inflated. Equilateral, beaks at the midline, not elevated, prosogyrate. Outline subcircular, length slightly greater than height; posterior dorsal (escutcheon) margin long, sloping steeply, almost straight, demarcating a narrow and shallow escutcheon. Posterior ventral, ventral and anterior margins forming a continuous curve to meet a short distinctly demarcated lunule. Posterior sinus indistinct, setting off a relatively wide posterior area. Ligament opisthodetic on a deeply sunken resilifer just over half the length of the escutcheon. Hinge teeth absent. Sculpture of irregular commarginal lines and growth stops, with a few distinct raised ridges, these increasingly spaced towards the ventral margin. Periostracum thin, persistent, straw coloured, microscopically spicate (Fig. 9C) and best preserved at the margins. The perisostracal spines are uncalcified. Internal muscle scars distinct, anterior adductor scar long and almost twice the area of the posterior scar, not separated from the pallial line; posterior adductor scar narrow, partly on the posterior area; pallial line thick, in distinct radial blocks, entire.

Gross anatomy (Fig. 9D). Mantle edge free except for a short siphonal opening posteriorly, inner fold thick and glandular. Anterior adductor muscle elongate slightly longer than posterior adductor muscle but twice as wide. Foot vermiform. Ctenidia with narrow outer and inner demibranchs, outer demibranch scarce-

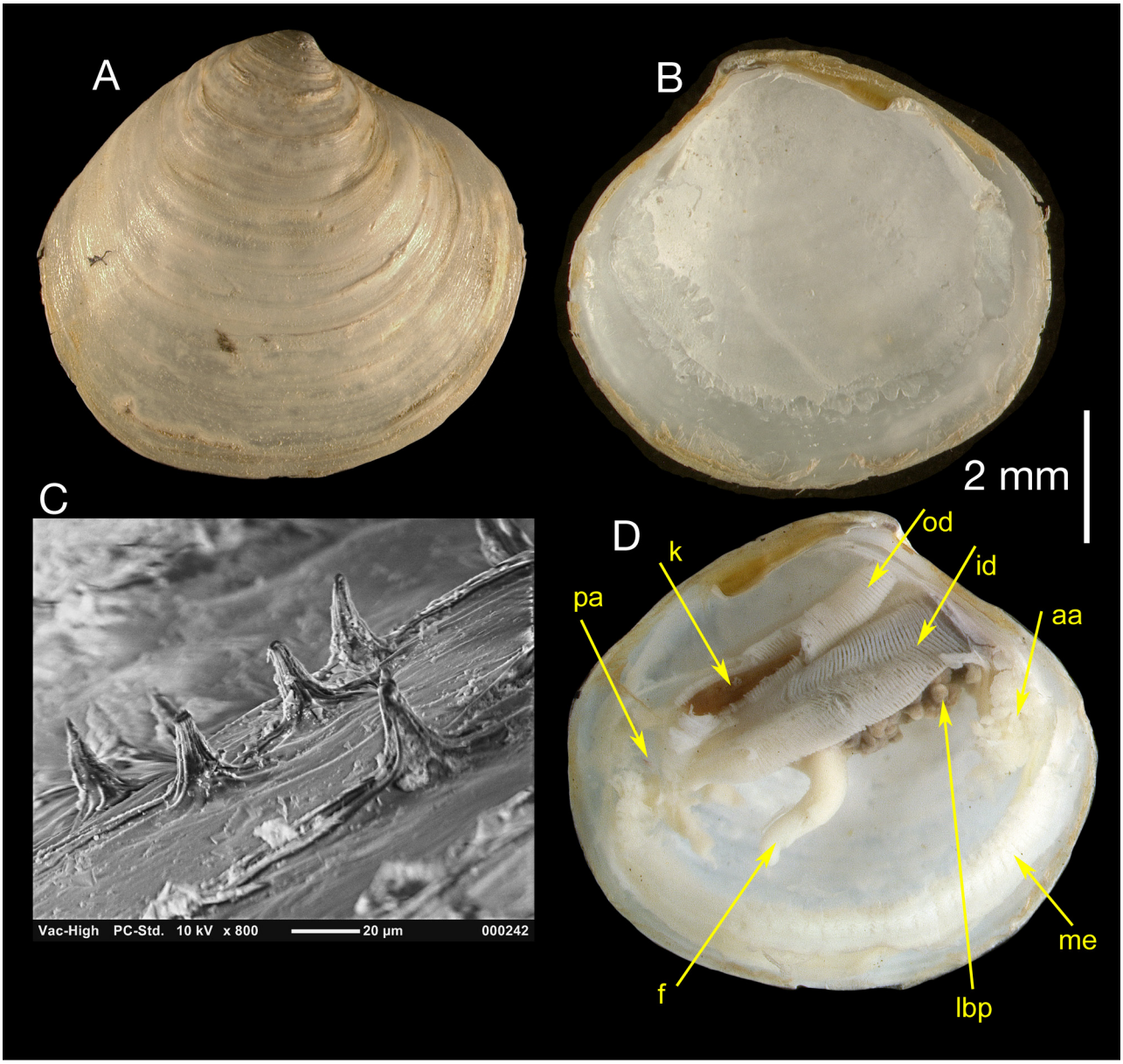

FIG. 9. – S*pinaxinus phrixicus* n. sp. Holotype. A exterior of right valve; B, interior of right valve; C, scanning electron micrograph of periostracal bristles; D, gross anatomy as viewed from th right side. aa, anterior adductor muscle; f, foot; id, inner demibranch; k, kidney; lbp, lateral body pouch; me, mantle edge; od, outer demibranch; pa, posterior adductor muscle.

ly overlapping inner; both with thick laminar reflected filaments. Labial palps narrow. Lateral body pouches relatively small, mostly hidden by ctenidia, tubules irregularly rounded. Kidneys large, bright orange in colour due to dense granular contents.

Etymology. From the Greek *phrixikos*, meaning "rippled" (from Brown 1956) and referring to the increasingly spaced commarginal ridges on the shell.

Differential diagnosis. *Spinaxinus phrixicus* is primarily distinguished from the Atlantic species by the presence of commarginal ridges on the shell. The periostracal bristles are of the same structure in all species of *Spinaxinus* but in *S. phrixicus* the bristles are very fine and scarcely distinguishable even under low magnification.

Anatomically all three species are similar but in *S. phrixicus* the lateral body pouches are relatively small and the tubules are rounded rather than cuboidal.

Molecular data for *S. phrixicus* (as Fiji Back Arc species) was obtained by Taylor *et al.* (2007) and there is a 99% similarity based on the 28S sequence with *S. emicatus*. Unfortunately, an 18S sequence could not be obtained, making the comparison with the data in Taylor *et al.* (2007) less informative at the species level. The morphological differences noted above are, in thyasirid terms, striking and could not be considered

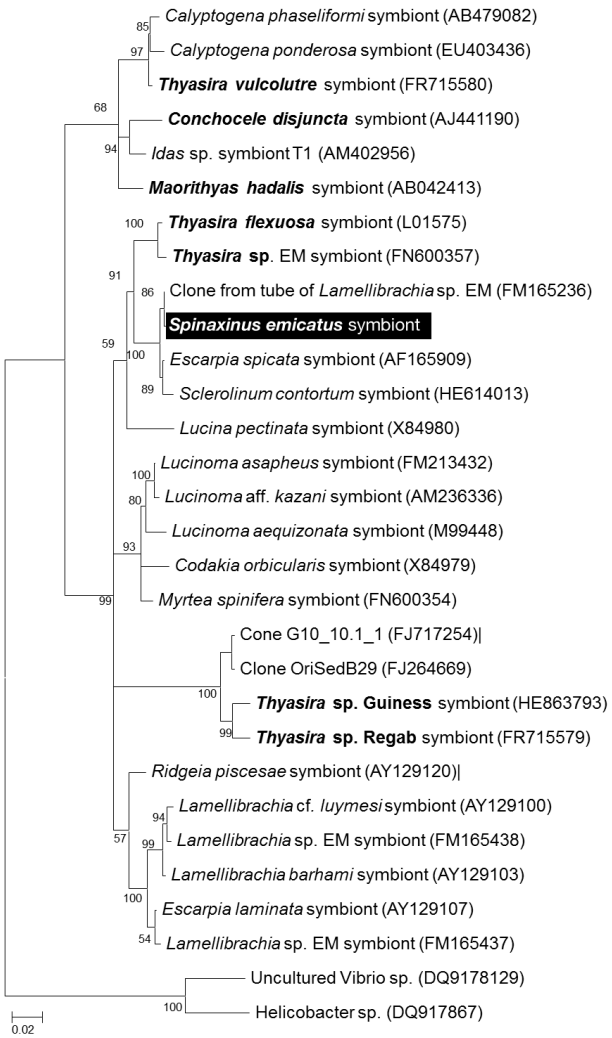

FIG. 10. – Phylogenetic tree based on the analysis of the bacterial 16 rRNA-encoding gene (1260 nucleotide positions analysed). The sequence from this study is in white on black and other thyasirid sequences are in bold. Bootstraps calculated from 500 NJ replicates (>50 shown). Scale bar represents 2% estimated divergence.

intra-specific. Biogeographically there is little in common between the Atlantic and Western Pacific bivalve faunas.

SYMBIOTIC BACTERIA

A single bacterial 16S rRNA phylotype was retrieved from three *Spinaxinus emicatus* specimens and presented 99% similarity (1336/1342 bp) with a bacterial phylotype found associated with the *Lamellibrachia* sp. tube from the eastern Mediterranean (FM165236). Highly similar sequences included gammaproteobacterial symbionts from the tubeworms *Sclerolinum contortum* and *Escarpia spicata*, and from the thyasirid *Thyasira flexuosa* and the lucinid *Anodontia fragilis* (95-98%). (Fig. 10)

Two phylotypes were obtained for APS reductase differing by 7 over 330 bp (no aminoacid differences), both showing 92% similarity to the APS reductase of *Thyasira* sp. eastern Mediterranean (FR715576).

DISCUSSION

The most intriguing aspect of this study is the sporadic occurrence of *Spinaxinus* in the Atlantic Ocean. *Spinaxinus* has now been retrieved from two artificial substrates but, with the exception of a single juvenile specimen, it has not yet been collected from other habitats, although habitats based on decaying organic matter have been poorly investigated to date (Samedi *et al*. 2010). The single juvenile thyasirid, tentatively identified as *S. sentosus,* came from the Gulf of Cadiz (Oliver *et al*. 2011), where the large numbers of thyasirids collected are, with this exception, *T. vulcolutre* (Rodrigues *et al*. 2008). Chemosymbiotic thyasirids have been recovered from other seep complexes such as those in the eastern Mediterranean (Rodrigues and Duperron 2011), West Africa (Duperron *et al.* 2012), the Gulf of Mexico (Cordes *et al*. 2007, 2009), Skagerrak (Dando *et al.* 2004) and Hakon Moseby (Gebruk *et al*. 2003), and are frequently found in reducing sediments throughout the Atlantic (Payne and Allen 1991, Oliver and Killeen 2002). Recent and current sampling efforts being made from seeps and vents (eg. Regab, Guiness and Hatton-Rockall) continue to produce undescribed thyasirids (Oliver and Drewery 2013) but *Spinaxinus* has never been recorded.

In the Pacific *Spinaxinus phrixicus* is associated with hydrothermal vents but in the Atlantic the only known vent species are *T. southwardae* from Anya's Garden (Oliver and Holmes 2006) and an undescribed fossil species from Rainbow (Lartaud *et al.* 2010). Vent sites are typically dominated by hard substrates where one would not expect infaunal deep burrowing species, such as thyasirids, to be commonly found.

A variety of organic falls produce reducing conditions and the association of Atlantic *Spinaxinus* with decaying vegetation may indicate such a natural setting. The faunal compositions of organic falls and those at vents/seeps are reported to be distinct (Bernardino *et al.* 2012), suggesting that different environmental markers are present. However, one well-studied thyasirid (*T. sarsi*) reveals that such conservatism is not universal. In Norwegian fjords *T. sarsi* has been found at sites with high organic enrichment from decaying vegetation or sewage (Dando and Southward 1986); in the Skagerrak from an active methane seep (Dando *et al.* 1994); in the North Sea from a weakly active pock mark (Dando *et al.* 1991); and in the North Sea associated with diesel contaminated drilling muds under oil platforms (Oliver and Killeeen 2002).

Faunas associated with organic falls have been the subject of a number of surveys and experiments, with that of Gaudron *et al.* (2010) deploying artificial traps, using alfalfa leaves along with wood and carbonate cubes. Unidentified juvenile thyasirids were retrieved from traps filled with wood but none from those with

alfalfa grass. The thyasirids were not unique to the traps but were known from the surrounding sediments (Gaudron *et al.* 2010). Small thyasirids, associated with woodfalls, are also reported by Bernardino *et al.* (2010) but not by Pailleret *et al.* (2007) or by Bienhold *et al.* (2013). Deep-sea settings influenced by macrovegetation input have been studied, including the Puerto Rico and Cayman trenches in the Caribbean, but hereto thyasirids have not been reported (Wolff 1979).

Whether *Spinaxinus* occurs on natural organic falls remains an open question, as very few data are available for comparison. It could be an early colonizer of natural organic falls (simulated by deployments), and might be replaced as the colonization succession proceeds.

Turning attention to the bacterial symbiont distribution, it is noted that *S. emicatus* symbionts belong to the Gammaproteobacteria and possess an APS reductase-encoding gene, which suggests a sulfur-linked metabolism. These symbionts are related to other thiotrophic bacteria associated with siboglinid tubeworms, and thyasirid and lucinid clams (Distel and Wood 1992; Rodrigues and Duperron 2011). Their 16S rRNA- and APS-encoding gene sequences are closely related to bacterial sequences found in the eastern Mediterranean cold seeps (Fig. 10) (associated with the tube of *Lamellibrachia* sp. and *Thyasira* sp. eastern Mediterranean, respectively). The *S. emicatus*–associated bacterial 16S rRNA sequence is more closely related to sequences from the tube and the trophosome of several tubeworms than to other thyasirid symbionts. Symbionts are most likely acquired from the environment in Thyasiridae, and several groups of Gammaproteobacteria have been reported as thyasirid symbionts (Rodrigues and Duperron 2011). It is thus possible that symbionts of *Spinaxinus* result from a host shift from tubeworm-associated bacteria, or from free-living relatives of these bacteria. Tubeworm symbionts are indeed reported to occur free-living in the environment, making such a host shift possible (Harmer *et al.* 2008), although this hypothesis remains to be properly tested here. The symbiont distribution as illustrated in Figure 10 shows no pattern of relationship with the thyasirid host, the environmental setting or the geographical location. Indeed, the *Spinaxinus* symbiont clusters with symbionts from deep cold seep species (siboglinids and bivalves) but also with a shallow water species (*Lucina pectina*) from reducing sediments. Other seep thyasirid symbionts cluster in two widely separated groups, one including species from the NE Atlantic and NW Pacific.

Symbionts are very abundant in *S. emicatus* and the gill is of Type 3, as described by Dufour (2005) and reported in species such as *T. methanophila* (Oliver and Sellanes 2005) and *Axinus cascadiensis* (Oliver and Holmes 2007). The structure and functioning of the symbiosis in *Spinaxinus* would therefore appear to be no different from that in several other thyasirids previously documented to harbour sulfur-oxidizing symbionts, and thus cannot explain the unusual restricted occurrences.

Artificial substrates were deployed to answer questions such as larval dispersion, community succession and organism growth rates of cold seep organisms (Carney *et al.* 2010). The appearance of previously unknown species suggests that the biogenerators do not entirely mimic natural cold seeps but represent an earlier stage of development of a reducing environment.

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